SALMONELLA ENTERICA SUBSP. *ENTERICA* 1,4,[5],12:i:-

Swine Health Information Center

SUMMARY

APRIL 2021

IMPORTANCE

- Nontyphoidal *Salmonella* are a leading cause of foodborne infections in humans. Animals are reservoirs for many salmonellae, including *Salmonella* 1,4,[5],12:i:-, an emerging serotype in swine.
- *S.* 1,4,[5],12:i:- is a monophasic variant of *Salmonella* Typhimurium. It has become one of the most identified serotypes in pigs, pork, and humans worldwide. Isolates are often resistant to multiple antimicrobials and heavy metals, making *S.* 1,4,[5],12:i:- a public health concern.

NOMENCLATURE

- The antigenic formula for each *Salmonella* serotype is based its subspecies and surface antigens: O (somatic), Vi (capsular, if present—but not found in *S.* Typhimurium), and H (flagellar).
- The antigenic formula *S.* 1,4,[5],12:i:- can be interpreted as follows:
  - *Salmonella* subspecies is *enterica*, designated by the number 1
  - O antigens are 4, [5], and 12; brackets around 5 indicate that is a variable epitope but the basis for variability is not known
  - Phase 1 H antigen (i) is present
  - Phase 2 H antigen (1,2) is absent, designated by a minus sign
- Serotypes that lack a full antigenic formula, including monophasic variants of *S.* Typhimurium, cannot be given a descriptive name (e.g., *Salmonella* Typhimurium, *Salmonella* Derby). Accordingly, for *S.* 1,4,[5],12:i:-, the antigenic formula is also the serotype name.

PUBLIC HEALTH

- *Salmonella* spp., including *S.* 1,4,[5],12:i:-, mainly cause gastroenteritis in humans. Less commonly, systemic disease and extra-intestinal infections occur.
- Incidence of *S.* 1,4,[5],12:i:- has increased dramatically in recent years, and many isolates from pigs, pork, and humans are highly related.
- The most common resistance phenotype is ASSuT, which involves resistance to ampicillin, streptomycin, sulfonamides, and tetracycline. Resistance to other antimicrobials has also been documented including quinolones, extended-spectrum β-lactams, phenicols, and colistin.
- Additionally, resistance to heavy metals including arsenic, copper, and silver (As/Cu/Ag) and mercury has been noted in recent *S.* 1,4,[5],12:i:- clones.

INFECTION IN SWINE

- The main serotypes associated with clinical salmonellosis in pigs are *S.* Typhimurium and *S.* 1,4,[5],12:i:-. Enterocolitis and septicemia can be seen. Most pigs recover but they may shed bacteria for months after resolution of clinical signs.
- Swine are also subclinical carriers of *S.* 1,4,[5],12:i:- and many other *Salmonella* serotypes. In carrier animals, shedding is exacerbated by stress including commingling, transport, and food deprivation.

TREATMENT

- Antimicrobial treatment is indicated for pigs with clinical signs and lesions suggestive of salmonellosis. Treatment regimens should be based on antibiograms, especially for emerging serotypes like *S.* 1,4,[5],12:i:- that often demonstrate multi-drug resistance.

CLEANING AND DISINFECTION

- *Salmonella* spp. can survive for long periods in the environment and are isolated from many sources. Salmonellae are generally susceptible to 1% sodium hypochlorite, 70% ethanol, 70% propanol, 2% glutaraldehyde, and 4% formaldehyde, as well as phenol, peracetic acid, hydrogen peroxide, quaternary ammonium compounds, and iodophors.
- A study of *S.* 1,4,[5],12:i:- in swine slaughterhouses found that isolates were susceptible to 0.5% peracetic acid, 1% peracetic acid, and 0.5% quaternary ammonium.

PREVENTION AND CONTROL

- To prevent shedding in carrier animals, reduce environmental and transport-related stress.
- Pens and contaminated fomites must be cleaned and disinfected thoroughly to reduce *Salmonella* load in the environment.
- Keep feed in rodent-proof containers to prevent contamination.
- Antimicrobials are not indicated for treatment of subclinical salmonellosis.

TRANSMISSION

- Most *Salmonella* transmission is fecal-oral. However, direct pig-to-pig transmission, inhalation, and vertical transmission also occur.

PATHOGENESIS

- *S.* 1,4,[5],12:i:- invades enterocytes, Peyer's patches, M cells, and goblet cells in the intestinal tract. Once it enters the lamina propria, it survives in macrophages and neutrophils, spreading to the mesenteric lymph nodes, spleen, and liver.
- Acute inflammation and endotoxemia lead to development of systemic signs and lesions.

DIAGNOSIS

- Culture and biochemical testing are used to identify *Salmonella*, but subtyping is necessary to determine serotype. Techniques used for subtyping include classical serotyping (White-Kauffman scheme), pulsed-field gel electrophoresis (PFGE), multiple locus variable number of tandem repeats (MLVA), multilocus sequence typing (MLST), and whole genome sequencing (WGS).
- Polymerase chain reaction (PCR) assays are not used for routine diagnosis, but several assays have been described that can differentiate *S.* 1,4,[5],12:i:- from *S.* Typhimurium.
- Enzyme-linked immunosorbent assays (ELISAs) may be useful for herd-level screening, and there are several commercial assays available based on lipopolysaccharide (LPS) O-antigen that can identify *Salmonella* to the serogroup level.
- For pigs with diarrhea, pooled ileum, colon, and ileocecal lymph node are preferred for culture. Feces or tonsil scrapings can be collected from live pigs. In cases of septicemia, blood, lung, liver, and spleen are acceptable. Most ELISAs can be used with serum or meat juice. In addition, oral fluids may be useful as a surveillance tool.

EPIDEMIOLOGY

- *S.* 1,4,[5],12:i:- occurs worldwide, and has been detected in cattle, chickens, and other birds in addition to pigs and humans.
- In recent years, *S.* 1,4,[5],12:i:- has become one of the top serotypes identified in clinical samples from pigs. *S.* 1,4,[5],12:i:- morbidity can be high, with diarrhea spreading rapidly throughout a pen. Mortality is generally low and associated with hypokalemia and dehydration following several days of diarrhea.

ETIOLOGY

- Salmonellae are motile, rod-shaped, Gram negative bacteria in the family *Enterobacteriaceae*. There are two main species, *Salmonella bongori* and *Salmonella enterica*.
- *S. enterica* is further classified into 6 subspecies, with *S. enterica* subsp. *enterica* being the most common. Subspecies are divided into more than 50 *Salmonella* serogroups based on O (somatic) antigen. These are further divided into serotypes based on H (flagellar) antigen. There are more than 2600 serotypes (also known as serovars) belonging to *S. enterica*.
- *S.* 1,4,[5],12:i:- is generally acknowledged as a monophasic variant of *S.* Typhimurium, lacking the phase 2 flagella genes *fljA* (first phase flagellin gene repressor) and *fljB* (second phase flagellin gene).

HISTORY IN SWINE

- To date, no major outbreaks of clinical salmonellosis due to *S.* 1,4,[5],12:i:- have been described in swine.

IMMUNITY

- Since *Salmonella* spp. are facultative intracellular pathogens, both IgA and cell-mediated immunity are a critical part of the response. Experimentally, seroconversion occurs from seven to 49 days after infection with *S.* 1,4,[5],12:i:-.
- Experimental vaccines have been described, but there is no commercial vaccine for *S.* 1,4,[5],12:i:-. No studies were found on cross-protection between *S.* 1,4,[5],12:i:- and other serotypes.

GAPS IN PREPAREDNESS

- *S.* 1,4,[5],12:i:- has emerged in both people and pigs as an important cause of salmonellosis. This trend may be partly due to changes in reporting practices and increased awareness, though it has been observed worldwide.
- The success of *S.* 1,4,[5],12:i:- may be due to factors including increased competitive fitness *in vivo*, enhanced survivability in feces, and better adaptation to the environment through antibiotic and heavy metal resistance.
- More information is needed to understand why and how *S.* 1,4,[5],12:i:- has become a dominant serotype in humans and pigs.

SALMONELLA ENTERICA SUBSP. *ENTERICA* 1,4,[5],12:i:-

Swine Health Information Center

LITERATURE REVIEW APRIL 2021

IMPORTANCE

Nontyphoidal *Salmonella* are a leading cause of foodborne infections in humans. Animals are reservoirs for many salmonellae, including *Salmonella enterica* subsp. *enterica* ser. 1,4,[5],12:i:- (abbreviated as *S.* 1,4,[5],12:i:-), an emerging serotype in swine. *S.* 1,4,[5],12:i:- is a monophasic variant of *Salmonella enterica* subsp. *enterica* ser. Typhimurium (*S.* Typhimurium). It has become one of the most identified *Salmonella* serotypes in pigs, pork, and humans worldwide. Isolates are often resistant to multiple antimicrobials and heavy metals, making *S.* 1,4,[5],12:i:- a public health concern.

NOMENCLATURE

Salmonella nomenclature is complex. A more detailed description can be found under *Etiology*. Briefly, the antigenic formula for a *Salmonella* serotype is based on its subspecies and surface antigens: O (somatic), Vi (capsular, if present), and H (flagellar, phase 1 and phase 2, if present). When writing the antigenic formula, a colon is placed between each antigen group. Accordingly, the antigenic formula for *S.* 1,4,[5],12:i:- can be interpreted as follows:

- *Salmonella* subspecies is *enterica*, designated by the number 1
- O antigens are 4, [5], and 12; brackets around 5 indicate that is a variable epitope but the basis for variability is not known
- Phase 1 H antigen (i) is present
- Phase 2 H antigen (1,2) is absent, designated by a minus sign

For serotypes that lack a full antigenic formula, like *S.* 1,4,[5],12:i:-, the antigenic formula becomes the serotype name. This contrasts with *S.* Typhimurium, for example, which has the antigenic formula *S.* 1,4,[5],12:i:1,2 and also has a descriptive name.

PUBLIC HEALTH

INFECTION IN HUMANS

Gastroenteritis is the most common presentation of salmonellosis in humans, characterized by nausea, vomiting, abdominal pain, and diarrhea. Systemic disease and extra-intestinal infections are less frequent, occurring mostly in people who are immunocompromised.[1]

S. 1,4,[5],12:i:- was first identified in the 1980s in poultry in Portugal.[2] Before the 1990s, it was rarely associated with illness in humans. However, *S.* 1,4,[5],12:i:- has now emerged as a leading cause of salmonellosis.[3] In the United States, the incidence of *S.* 1,4,[5],12:i:- has increased 580% since 2001, and it has been among the top five *Salmonella* serotypes reported to the Laboratory-based Enteric Disease Surveillance (LEDS) system since 2011.[4] In Europe, it is one of three main serotypes in pigs and pork meat that are associated with human illness (as cited by Campos *et al*).[5]

Additionally, *S.* 1,4,[5],12:i:- has become a top serotype isolated from pigs and pork (see *Epidemiology*). Pigs often carry *Salmonella* subclinically. However, fecal shedding is exacerbated by stress, and prevalence of infection increases with

the amount of time spent in lairage. Fecal contamination during slaughter is the main source of *Salmonella* (including *S.* 1,4,[5],12:i:-) in pork.[6, 7]

Multiple studies have shown that *S.* 1,4,[5],12:i:- isolates from pigs, pork, and humans are highly related.[5, 8] One US outbreak involved pork meat from Washington state.[9] *S.* 1,4,[5],12:i:- outbreaks have also been linked to pot pies,[10] alfalfa sprouts,[11] frozen shredded coconut,[12] kratom,[13] and kosher chicken,[14] as well as frozen rodents used for reptile feed.[15]

Table 1 describes outbreaks of salmonellosis in humans linked to pork.

Table 1. Outbreaks of *S.* 1,4,[5],12:i:- Linked to Pork*

Location	*Source*	*Year*	*Cases*	*Deaths*
Luxembourg[16]	Pork meat	2006	63	1
France[17]	Dried pork sausage	2010	69	0
France[18]	Dried pork sausage	2011	337	0
Italy[19]	Pork salami	2012-15	--	--
Spain[20]	Pork chorizo	2014	6	0
Spain[21]	Dried pork sausage	2011	38	0
United States[9]	Pork meat**	2015	192	0
Germany[22] O4 non-agglutinating monophasic *S.* Typhimurium variant	Minced pork	2015	61	0
Sweden	Italian chilled truffle salami	2018	--	--
Spain[23]	Roast pork	2018	112	0

*Adapted from Campos *et al* 2019

**Recalled products included whole pigs for barbeque, various pork offal products, pork blood, and pork trim.

ANTIMICROBIAL RESISTANCE

Multi-drug resistant (MDR) *S.* 1,4,[5],12:i:- clones are frequently detected, particularly the ASSuT phenotype, which is resistant to ampicillin, streptomycin, sulfonamides, and tetracycline.

- In 2018, the National Antimicrobial Resistance Monitoring System (NARMS) found that 23% of MDR *Salmonella* from humans were serotype *S.* 1,4,[5],12:i:-. Of those, 92% were the ASSuT phenotype. One *S.* 1,4,[5],12:i:- isolate was categorized as extremely resistant (resistant to eight or more antimicrobial classes).[24] MDR *S.* 1,4,[5],12:i:- isolates were also identified very frequently in cecal samples from market swine.
- The 2017-18 European Union Summary Report on Antimicrobial Resistance identified high MDR levels in *S.* 1,4,[5],12:i:- from humans (81.4%), pig carcasses (77.2%) and pigs (78.9%).[25] Most isolates, from all three categories, were resistant to ampicillin, sulfamethoxazole, and tetracycline.[25]

Three resistance regions (RR 1, 2, 3) confer the ASSuT phenotype. RR 1 and 2 are surrounded by intersequence (IS) *26* elements and highly similar to a region of plasmid pO111_1 from *Escherichia coli* (which carries a mercury resistance operon). RR3 replaces DNA located between *STM2759* and *iroB*, including the *fljBA* operon and contains the antimicrobial resistance (AMR) genes *bla*-$_{TEM}$, *strA*-*strB*, *sul2*, and *tet*(B) on an IncH1 plasmid.[26] Resistance mechanisms are briefly described below.

- *β-lactams*: resistance conferred by horizontally acquired β-lactamases encoded by *bla*$_{TEM-1}$ and *bla*$_{PSE-1}$, for example, which cause ampicillin resistance.[27]
- *Aminoglycosides*: resistance usually due to acetyltransferases, phosphotransferases, and nucleotidyltransferases which modify and inactivate the drug (encoded by *aac*, *aad*, *aph*, and *str* genes and their variants).[27]

- *Folate pathway inhibitors*: resistance due to acquisition of genes encoding enzymes that prevent binding of dihydropteroate synthase (sulfonamides, encoded by *sul1*, *sul2*, *sul3*) and dihydrofolate reductase (trimethoprim, encoded by *dhfr, dfr*).[27]
- *Tetracyclines*: resistance mechanisms include active efflux, encoded by *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(G), and *tet*(H); modification of rRNA targets; and compound inactivation.[27]

Resistance to additional antimicrobials including quinolones, extended-spectrum β-lactams, colistin, and phenicols has also been documented in isolates from pigs and pork (as well as other species and humans, in many cases). Resistance mechanisms are briefly described below.

- *Quinolones*: resistance mechanisms include mutation in the *gyrA–gyrB* and *parC–parE* gene pairs; acquired plasmid-mediated quinolone resistance (PMQR) genes including *qnrS1, qnrB19,* and *aac(6')Ib-cr*; and altered expression of efflux pumps and porin diffusion channels. Resistant *S.* 1,4,[5],12:i:- isolates have been documented in pigs[28-30] and pork.[31]
- *Extended-spectrum β-lactams*: resistance conferred by horizontally acquired β-lactamases encoded by *bla*-$_{\text{CTX-M-1}}$, *bla*-$_{\text{CTX-M-14}}$, *bla*-$_{\text{CTX-M-15}}$+bla$_{\text{SHV-12}}$, and *bla*-$_{\text{CTX-M-32}}$, among others. Resistant *S.* 1,4,[5],12:i:- isolates have been found in pigs[29, 32, 33] and pork.[34, 35]
- *Colistin*: resistance conferred by plasmid-mediated *mcr-1* (most identified type in swine, there are other *mcr* variations). Resistant *S.* 1,4,[5],12:i:- isolates have been found in pigs,[36-38] pork,[39] and swine at slaughter.[5, 40]
- *Phenicols*: resistance mechanisms include inactivation of efflux pumps (encoded by *floR, cmlA*) and inactivation of chloramphenicol acetyltransferase (encoded by *cat1, cat2*).[27] Resistant *S.* 1,4,[5],12:i:- isolates have been found in pigs and pork.[41-43]

HEAVY METAL RESISTANCE

Resistance to heavy metals has been documented among *S.* 1,4,[5],12:i:- isolates from the UK/Europe,[44-46] the United States,[29] and Canada.[47] *Salmonella* genomic island-4 (SGI-4, previously identified as SGI-3)[44] encodes tolerance to arsenic, copper, and silver (As/Cu/Ag) and RR1 and 2 (also known as the mercury resistance element, MREL) encode tolerance to mercury. Both SGI-4 and MREL are thought to be mobile genetic elements.

The 2015 US pork outbreak isolate, USDA15WA-1, contained SGI-4 and an MDR module inserted in the *fljB* region, which encoded tolerance to mercury and antimicrobial resistance to ampicillin, streptomycin, sulfisoxazole, and tetracycline.[48] A further experimental study confirmed that exposure to zinc and copper (as an antimicrobial feed additive) led to the induction of multiple metal tolerance genes (copper, arsenic, silver, and mercury) in USDA15WA-1.[49]

INFECTION IN SWINE

Pigs are most often infected with *Salmonella* spp. asymptomatically, shedding bacteria in their feces continually or intermittently, for long periods of time. Stress, such as commingling, transport, and food deprivation, can exacerbate shedding.[6]

In pigs, salmonellae can cause enterocolitis and septicemia. Diarrhea may last for three to seven days initially, then recur over several weeks. Feces are usually yellow and watery and may contain blood sporadically. Fever and anorexia are also common. Clinical infection with serotypes other than *S.* Typhimurium or *S.* 1,4,[5],12:i:- is uncommon.[6]

S. 1,4,[5],12:i:- has been increasingly isolated from pigs in recent years (see *Epidemiology*). Clinical signs of *S.* 1,4,[5],12:i:- are indistinguishable from *S.* Typhimurium, and studies have shown that the pathogenic potential of these serotypes is similar, as described below.

- A study published in 2014 found that adhesion and invasion of porcine intestinal epithelial cells (PIEC-1) was comparable for *S.* 1,4,[5],12:i:- and *S.* Typhimurium.[50]
- In 2017, clinical submissions (enteric samples) from pigs 3 to 13 weeks-of-age were reviewed at a diagnostic laboratory.[51] A positive association was found between isolation of *S.* 1,4,[5],12:i:- and histologic lesions consistent with enteric salmonellosis.[51]
- In 2018, pigs were inoculated with the 2015 pork outbreak isolate USDA15WA-1 to assess pathogenicity. Slight fever and diarrhea were seen at two days post-infection (dpi). Fecal shedding occurred throughout the 7-day study, and colonization of intestinal tissues was also documented.[52]
- Tissue colonization by *S.* 1,4,[5],12:i:- (tonsils, mesenteric lymph nodes, and intestinal contents) was similarly confirmed in experimentally infected piglets in 2019.[53]
- A 2019 study compared experimental infection of pigs with *S.* Derby, *S.* Typhimurium, and *S.* 1,4,[5],12:i:-. Diarrhea was seen in pigs inoculated with *S.* 1,4,[5],12:i:-, but fever did not occur. Fecal shedding was greatest for pigs with *S.* 1,4,[5],12:i:-, compared to the other groups, and occurred continuously during the trial.[54]
- An additional 2019 study compared pathogenicity of *S.* 1,4,[5],12:i:-, *S.* Typhimurium, and *S.* Derby, confirming that *S.* 1,4,[5],12:i:- causes clinical disease in inoculated pigs (fever, diarrhea), fecal shedding, and tissue colonization. Gross and histologic lesions suggestive of salmonellosis were also seen in pigs inoculated with *S.* 1,4,[5],12:i:-. Furthermore, to evaluate competitive fitness, pigs were co-inoculated with *S.* 1,4,[5],12:i:- and *S.* Typhimurium. *S.* 1,4,[5],12:i:- was detected in the feces of more pigs, at higher levels, compared to *S.* Typhimurium. In addition, superior fitness was seen for *S.* 1,4,[5],12:i:- regarding colonization of the tonsils and ileocecal lymph nodes.[55]

Gross and histopathologic lesions associated with *S.* 1,4,[5],12:i:- include fibrinous colitis (especially in the spiral colon), mesenteric lymphadenopathy, and neutrophil infiltration, crypt elongation, and erosion or ulceration of the cecum and spiral colon.[51, 55] However, the development of clinical disease and gross lesions is known to vary among individuals.[56, 57] Absence of lesions does not mean absence of infection.

TREATMENT

Generally, mass medication may be used during *Salmonella* outbreaks to decrease the severity of disease and transmission of bacteria.[6] Historically, amikacin, gentamicin, apramycin, ceftiofur, and trimethoprim/sulfonamide have been effective *in vitro* against most salmonellae (as cited by Griffith *et al*).[6] However, treatment regimens should be based on antibiograms, especially for emerging serotypes like *S.* 1,4,[5],12:i:- that often demonstrate multi-drug resistance (see *Public Health*). In an outbreak situation, oral antimicrobials (through feed or water) may prevent disease in susceptible pigs that are not yet infected.[6] Anti-inflammatory drugs like flunixin and meloxicam are also appropriate.[6]

CLEANING AND DISINFECTION

SURVIVAL

Salmonella spp. can survive for long periods in the environment and are isolated from many sources. *S.* Choleraesuis survives for at least three and 13 months in wet and dry swine feces, respectively.[58] In organic pigs raised outdoors, *S.* Typhimurium survives for up to five weeks in soil and seven weeks in shelter huts.[59] In swine feces inoculated with *Salmonella* spp., survival was documented for up to 88 days.[60] Supplementation of swine diets with organic acids has been shown to reduce fecal *Salmonella* load.[61-63] However, in pigs fed diets supplemented with organic acids, *S.* Typhimurium DT193 and *S.* 1,4,[5],12:i:- survived longer than other serotypes (*S.* Typhimurium DT104b, *S.* Derby and *S.* Bredeney) due to their adaptation to low fecal pH.[60]

Most *Salmonella* spp. are sensitive to heat and are killed at temperatures greater than 70°C. The optimal pH range is between 6.5 and 7.5, though *Salmonella* may grow from pH 4 to 9.[64]

DISINFECTION

Generally, salmonellae are susceptible to many disinfectants including 1% sodium hypochlorite, 70% ethanol, 70% propanol, 2% glutaraldehyde, and 4% formaldehyde, as well as phenol, peracetic acid, hydrogen peroxide, quaternary ammonium compounds, and iodophors.[1] They can also be killed by moist heat (121°C) for a minimum of 15 minutes or dry heat (170°C) for at least one hour.[1]

In a study of swine slaughterhouses, *S.* 1,4,[5],12:i:- was isolated from holding pens before and after cleaning, and from a carcass at the bleeding table. All samples were susceptible to 0.5% peracetic acid, 1% peracetic acid, and 0.5% quaternary ammonium.[65]

PREVENTION AND CONTROL

DISEASE REPORTING

Salmonellosis is not an OIE-listed disease in pigs.

DISEASE PREVENTION

Carrier pigs are an important source of infection. However, the value of serological testing is unclear since antibody response and shedding on the farm are not predictive of *Salmonella* isolation at slaughter.[66, 67]

Stress exacerbates fecal shedding and suppresses the immune system of susceptible pigs.[6] To minimize stress producers should prevent commingling and reduce environmental and transport-related stress. Specific recommendations include:

- Fill grower/finisher rooms with single-source, single-age pigs, and do not over-stock
- Keep pens dry and comfortable, ensuring proper temperature and adequate ventilation
- Do not transport sick or injured pigs, do not transport during extreme temperatures or inclement weather, and avoid overcrowding on trucks
- Do not hold pigs for extended times in lairage

Antimicrobials are not indicated for treatment of subclinical salmonellosis.[68] There is no commercial vaccine for *S.* 1,4,[5],12:i:- (see *Immunity*).

Feed can be contaminated with *Salmonella*, including *S.* 1,4,[5],12:i:-, at the mill or on the farm.[69, 70] Keep feed in rodent-proof containers, and put a rodent control program in place.

DISEASE CONTROL

Since pigs with diarrhea contaminate their environment, and serve as a source of infection for others, removal and isolation are critical.[6] Pens and contaminated fomites must be thoroughly cleaned and disinfected. Other standard biosecurity practices should be in place to prevent the introduction of *Salmonella* spp. into clean herds, including shower-in/shower-out and visitor restriction. Producers should also acquire replacement animals and semen from *Salmonella*-free herds.[68]

Additionally, feed type can influence *Salmonella* prevalence on an infected farm. Recommendations are for meal (vs. pellets), coarse feed (vs. fine feed), and fermented liquid feed (vs. dry feed).[68] Acidification of feed and water may decrease *Salmonella* prevalence in infected herds. For example, organic acid supplementation of feed for two to three months before slaughter has been associated with reduced *Salmonella* seroprevalence, prevalence in mesenteric lymph nodes, and fecal shedding.[71] However, this practice may be less effective for pathogenic serotypes in swine since *S.* Typhimurium DT193 and *S.* 1,4,[5],12:i:- appear to be adapted to low fecal pH (see *Cleaning and Disinfection*).[60]

TRANSMISSION

Most transmission of salmonellae is fecal-oral.[6] *Salmonella* spp. are shed in the feces by both animals that are clinically ill and asymptomatic carriers, and the environment can be heavily contaminated. Feeds are also a potential source of bacteria. Pig-to-pig transmission is possible since the tonsils become colonized and oropharyngeal secretions contain bacteria.[6] Inhalation of aerosolized secretions, feces, and dust can also occur, as well as vertical transmission.[6]

Salmonella shedding is highly variable, and influenced by environment, feeding, and management practices.[6] Stress can induce shedding in subclinical carriers within hours. Following experimental infection, *S.* Typhimurium can be detected in feces for four to five months and tissues (mesenteric lymph node, tonsil, cecum) for four to seven months.[6] In a study of Australian pigs, shedding of *S.* 1,4,[5],12:i:- was documented for up to a year in pooled fecal samples following experimental inoculation.[72]

Antibiotics do not seem to reduce or prolong the duration and magnitude of shedding in pigs with enterocolitis; however, administration of antibiotics is associated with a prolonged carrier state in humans.[6]

PATHOGENESIS

Generally, invasion is mediated by a serotype-specific plasmid.[6] Portals of entry include Peyer's patches, enterocytes, M cells, and goblet cells. As described by Griffith *et al*,[6] attachment of the bacteria to epithelial receptors triggers microfilament-controlled update, followed by vacuole formation and transport through the cytoplasm. Entry into the lamina propria occurs via exocytosis through the basement membrane. There, bacteria survive in macrophages and neutrophils and spread to the mesenteric lymph nodes, spleen, and liver. For more information on adhesion, invasion, cytotoxicity, and resistance to intracellular killing, see *Major Virulence Determinants*.

Acute inflammation is a key factor of enteric salmonellosis, mediated by neutrophil recruitment and chemokine-promoted transmigration across the epithelium, as well as other proinflammatory agents.[6] As bacteria disseminate, endotoxemia leads to development of systemic signs and lesions.

DIAGNOSIS

CULTURE AND IDENTIFICATION

Salmonellosis is diagnosed by culture and identification in pigs with suggestive lesions.[6]

Samples from suspected cases of salmonellosis may undergo non-selective pre-enrichment, and then selective enrichment (with inhibitory reagents such as tetrathionate) prior to plating with selective media like MacConkey agar, Salmonella-Shigella (SS) agar, xylose lysine deoxycholate (XLD) agar, Hektoen enteric agar, and brilliant green agar.[64]

Presumptive *Salmonella* colonies are screened with biochemical test media like triple sugar iron agar (TSI), lysine iron agar (LIA), urea agar, motility-indol-ornithine agar (MIO), and Simmons citrate agar. *Salmonella* spp. also produce hydrogen sulfide (H2S).[64]

Chromogenic media (e.g., SM-ID agar, BBL CHROMagar *Salmonella*) have been developed that allow detection, enumeration, and identification directly on the plate. These media are more specific than conventional agars like XLD, but they do not offer increased sensitivity.[73]

SALMONELLA SUBTYPING

Serotyping, using the White-Kauffman scheme, is often the first step in characterizing *Salmonella* isolates. Serotype is determined by combining the isolate with somatic (O) and flagellar (H) antisera and checking for agglutination (see *Characteristics of Salmonellae*). This and other *Salmonella* subtyping methods are compared in Table 2 (note: applications stated are in the context of improving subtyping for *Salmonella* control in food production).

Table 2. Comparison of Common *Salmonella* Subtyping Methods*

Method	*Predictive ability*	*Discriminative ability*	*Time to results (from single colony)*	*Cost (reagents)*	*Main applications*
Classical serotyping (White-Kauffman)	Fair	Poor	2-17 days	$	Rapid confirmation and subtype screening**
Pulsed-field gel electrophoresis (PFGE)	Good	Good	4-6 days	$	Gold standard for *Salmonella* subtyping**
Multiple locus variable number of tandem repeats analysis (MLVA)	Good	Good	1-2 days	$	Secondary method for serotyping and PFGE**
Multilocus sequence typing (MLST)	Good	Fair	1-2 days	$$	Rapid confirmation and subtype screening**
Whole genome sequencing (WGS)	Excellent	Excellent	3-17 days	$$ to $$$	Useful for high demand situations, information on virulence genes and antibiotic resistance genes can be retrieved from data

*Adapted from Tang *et al*, 2019[74]

**Likely to be replaced by WGS serotype prediction

TESTS TO DETECT NUCLEIC ACIDS OR ANTIGEN

Polymerase chain reaction (PCR) assays are not used for routine diagnosis in pigs but may be valuable for screening. A few assays have been developed to differentiate *S.* 1,4,[5],12:i:- from *S.* Typhimurium as described:

- A protocol that combines traditional serotyping with multiplex PCR based on the region between *fljB* and *fljA*.[75, 76]
- Multiplex RT-PCR to detect *fliC* (present in many *Salmonella*), *fljB.1,2* (present in *S.* Typhimurium, absent in *S.* 1,4,[5],12:i:-) and *fliB/IS200* (present in both).[77]
- Multiplex oligonucleotide ligation-PCR to detect 52 molecular markers including prophage genes, amplified fragment length polymorphism elements, SGIs, allantoinase gene *allB*, MLVA locus STTR10, antibiotic resistance genes, single nucleotide polymorphisms (SNPs) and *fljB*.
- Multiplex RT-PCR to detect *invA* (present in all *Salmonella*); *fliA* (present in *S.* Typhimurium and *S.* 1,4,[5],12:i:-); *fljB*; intergenic space between *hin* and *iroB* (present in *S.* Typhimurium, may be absent in *S.* 1,4,[5],12:i:-).[78]

TESTS TO DETECT ANTIBODY

Enzyme-linked immunosorbent assays (ELISAs) are available but generally lack sensitivity and specificity needed for individual animal diagnosis.[6] They may be useful for herd-level screening. Commercial ELISAs use lipopolysaccharide (LPS) O-antigen to identify serogroups B, C1, and D (e.g., IDEXX Swine *Salmonella* Ab Test®, IDEXX, France; PrioCHECK™ Porcine *Salmonella* Kit, Thermo Fisher Scientific, USA).

SAMPLES

For pigs with diarrhea, pooled ileum, colon, and ileocecal lymph node are preferred for culture, but tonsil and cecal wall can also be used. Feces or pharyngeal tonsil scrapings can be collected from live pigs.[6] In cases of septicemia, blood, lung, liver, and spleen are acceptable.[6]

Most commercial ELISAs can be used with serum or meat juice. Oral fluids have been successfully tested with ELISAs and appear to be prospective tool for *Salmonella* surveillance.[79, 80]

EPIDEMIOLOGY

SPECIES AFFECTED

S. 1,4,[5],12:i:- is not host-specific like some *Salmonella* serotypes. It has been detected in cattle,[81, 82] chickens,[82, 83] and other birds[82] in addition to pigs and humans (see *Public Health*).

GEOGRAPHIC DISTRIBUTION

S. 1,4,[5],12:i:- has been reported in Asia, Europe, North America, South America, and Oceania.[26, 84]

MORBIDITY AND MORTALITY

S. 1,4,[5],12:i:- has increasingly been isolated from pigs in recent years.[78, 81, 85] A review of 10,194 clinical swine cases from 2008-2017 at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) found that *S.* 1,4,[5],12:i:- was the 13th most identified serotype in 2008. By 2017, *S.* 1,4,[5],12:i:- was the top serotype identified, and it made up one third of all clinical submissions. A corresponding decrease was seen in identification of *S.* Typhimurium and other serogroup B serotypes (e.g., *S.* Derby, *S.* Agona, *S.* Heidelberg).[78]

S. 1,4,[5],12:i:- morbidity can be high, with diarrhea spreading rapidly throughout a pen.[6] Mortality is generally low, and associated with hypokalemia and dehydration following several days of diarrhea.[6]

ETIOLOGY

CHARACTERISTICS OF SALMONELLAE[1, 26, 64, 86]

Salmonellae are motile, rod-shaped, Gram negative bacteria in the family *Enterobacteriaceae*. There are two broad species: *Salmonella bongori* and *Salmonella enterica*. Additionally, *S. enterica* is classified into six subspecies, with *S. enterica* subsp. *enterica* being the most common. Subspecies are divided into more than 50 *Salmonella* serogroups based on O (somatic) antigen. These are further divided into serotypes based on H (flagellar) antigen. There are more than 2600 serotypes (also known as serovars) belonging to *S. enterica.*

The antigenic formula for *Salmonella* spp. is composed of subspecies name: O antigens: Vi (capsular) antigens, if present: H antigens (phase I): H antigens (phase 2, if present). Salmonellae express either one or two H antigens (FliC and FljB) and are designated as monophasic or biphasic, respectively.

Additionally, salmonellae can be classified by phage type and MLST. Phage typing involves the ability of a particular phage to lyse a particular *Salmonella* strain. There are more than 300 known phage types. When used with antimicrobial susceptibility analysis, phage typing has been used to detect *S.* Typhimurium outbreaks (as cited by Ferrari *et al*).[8] The seven housekeeping genes used for MLST are *aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*.[87] Virulence genes (e.g., *fljB* and *fliC*) can be added to an MLST scheme.[8]

Virulence-related genes occur in clusters of chromosomes and plasmids known as *Salmonella* pathogenicity islands (SPIs). There are more than 20 known SPIs[88] and 200 known virulence factors associated with salmonellae.[6] SPI-1 and SPI-2 co-encode T3SS, a secretion system involved in invasion and dissemination through the transfer of effectors from bacteria to the host cytoplasm. The roles of 41 SPI-1 and SPI-2 effectors (proteins) produced by *S.* Typhimurium have been summarized by Wang *et al*.[89] Additionally, Ilyas *et al*. have summarized virulence factors related to bacterial survival and replication that are associated with other SPIs.[90]

CHARACTERISTICS OF *S.* 1,4,[5],12:i:-[26, 43, 82, 91-94]

S. 1,4,[5],12:i:- is generally acknowledged as a monophasic variant of *S.* Typhimurium, lacking the phase 2 flagella genes *fljA* (first phase flagellin gene repressor) and *fljB* (second phase flagellin gene). This relationship has been confirmed by methods including sequencing of virulence genes, PFGE, MLST, microarray analysis, whole genome sequencing, and comparison of antibiotic resistance patterns (as cited by Sun *et al*.).[26] Partial deletions and mutations of *fljB* and *hin*, which help regulate expression of flagellin genes, have been observed in some atypical monophasic variants.

Genetic analyses suggest that *S.* 1,4,5,12:i:- strains are continually emerging and many clones exist. Three main clones (Spanish, United States, and European) have previously been identified (shown in Table 3).[26] However, early isolates were characterized mainly by phenotype and molecular markers, and recent genetic analyses have led to identification of new *S.* 1,4,5,12:i:- clones.[29, 44, 93, 95]

Elnekave *et al*. suggest that *S.* 1,4,[5],12:i:- forms 2 clades regardless of source and geographic origin. U.S. swine isolates collected from 2014-15 were nearly identical to the "European" clade (ASSuT phenotype) vs. local (U.S./American clade) *S.* Typhimurium.[29]

Table 3. Comparison of *S.* 1,4,[5],12:i:- Clones from Pigs

	Phage type	*MLST type*	*AR pattern*	*Comparative genetic features*	*Main region*	*Year*
Spanish	U302	ST19	ACSSuT-GSxT	Variable deletions in fljAB operon, absence of Fels-1 and Fels-2 prophages, mutation of Gifsy-1 prophage, deletion of allantoin operon	Europe	1997-
US	--	--	-- (MDR rare)	Large deletion in fljAB operon, absence of Fels-2 prophage	United States	2004-
European	DT193 DT120	ST34	ASSuT	Deletions in fljAB operon, acquisition of SGI-4 (heavy metal resistance) and virulence gene sopE via prophage mTmV	Europe	2005-

*Adapted from Sun *et al*[26]

There is a higher diversity of phage types among *S.* Typhimurium isolates compared to *S.* 1,4,[5],12:i:-.[8, 77] Virulence determinants of *S.* 1,4,[5],12:i:- are very similar to those of *S.* Typhimurium.[26] They include:

- Virulence genes *gipA, sodC1, sopE1*, and *sspH1* (located on prophages) and *spvC, pefA,* and *rck* (located on a virulence plasmid)
- Toxin-antitoxin cassettes, such as type II TA, which enhances plasmid-mediated colistin resistance (via *mcr-1*)
- Biofilm formation, which can be enhanced by the presence of other gastrointestinal conditions, and leads to increased persistence in the environment

HISTORY IN SWINE

To date, no major outbreaks of clinical salmonellosis due to *S.* 1,4,[5],12:i:- have been described in swine.

IMMUNITY

POST-EXPOSURE

Although *Salmonella* spp. are facultative intracellular pathogens, there is a strong humoral response to natural infection, including secretion of IgA to prevent mucosal invasion.[96] Cell-mediated immunity (CMI) is also a critical part of the anti-*Salmonella* response.

- In a study of piglets experimentally infected with *S.* Typhimurium, *S.* 1,4,[5],12:i:-, and *S.* Derby, seroconversion occurred at days 28, 31, and 38 post-infection, respectively.[54]
- In 7-week-old pigs inoculated with *S.* 1,4,[5],12:i:-, antibodies were detected in 21% (5/24) of pigs at 7 dpi, and in 100% of pigs at 49 dpi.[53]

VACCINES

Salmonella vaccination has been used to control clinical disease when outbreak strains are matched to vaccine serotypes.[96] Stimulation of mucosal immunity and use of live vaccines and adjuvants to enhance cell-mediated immunity can improve vaccine efficacy.[96] Ideally, vaccines could be used to reduce or eliminate *Salmonella* carriage and shedding by the time of slaughter. A recent meta-analysis found a moderate effect of vaccination on the prevalence of *Salmonella* colonization and excretion in pigs regardless of vaccine type (attenuated or killed).[97] Vaccination is complicated by the large number of *Salmonella* serotypes that are found in swine.[96, 97]

Vaccines are available for *S.* Typhimurium in swine. Formulations to protect against *S.* 1,4,[5],12:i:- have been described experimentally. A live vaccine reduced the incidence and severity of disease in weaners,[98] and an autogenous inactivated vaccine for sows and piglets reduced colonization and fecal shedding.[99] However, there is no commercial vaccine for *S.* 1,4,[5],12:i:-.

CROSS-PROTECTION

Live vaccines may confer cross-protection against different *Salmonella* serogroups.[96] For example, pigs administered a live *S.* Choleraesuis vaccine (Argus SC) had reduced prevalence of *Salmonella* in the lymph nodes.[100]

To date, no studies have been published on cross-protection between *S.* Typhimurium and *S.* 1,4,[5],12:i:-.

GAPS IN PREPAREDNESS

S. 1,4,[5],12:i:- has emerged in both people and pigs as an important cause of salmonellosis. This trend may be partly due to changes in reporting practices and increased awareness, though it has been observed worldwide. The success of *S.* 1,4,[5],12:i:- may be due to factors including increased competitive fitness *in vivo*, enhanced survivability in feces, and better adaptation to the environment through antibiotic and heavy metal resistance. However, more information is needed to understand why and how *S.* 1,4,[5],12:i:- has become a dominant serotype in humans and pigs.

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